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Effects of two weeks administration of *Ocimum gratissimum* leaf on feeding pattern and markers of renal function in rats treated with gentamicin

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ABSTRACT

This study investigated the effects of aqueous extract of *Ocimum gratissimum* leaf (AOGL) on some markers of renal function in rats with gentamicin-induced nephropathy. Thirty adult male Wistar rats were used for this study. They were divided into 5 groups as follows: Group 1 (the control) (n = 5) received distilled water daily by oral route for the whole period of the study. Group 2 (the toxic control) (n = 10) received 100 mg/kg/day of gentamicin i.p. for a week. Groups 3, 4, and 5 (n = 5) were pre-treated with gentamicin as the Group 2 rats, after which they received 100, 200 and 400 mg/kg/day each of AOGL p.o., respectively, for 14 days. Rats in each group were placed inside separate metabolic cages to obtain their food consumption, water intake and urine output for 24 hours after the last administration. Markers of renal function such as creatinine, urea and total protein were determined both in the plasma and urine. Oxidative stress markers such as TBARS and GSH were assayed in the tissue homogenate. Creatinine clearance was calculated using a standard formula. Gentamicin treatment induced significant ($p < 0.05$) increases in urine output, plasma urea, creatinine, urinary protein, relative kidney weight and TBARS in the toxic control when compared to the control group. Significant decreases ($p < 0.05$) in urine creatinine and GSH were also associated with gentamicin administration. Post-treatment with AOGL caused significant increases in food consumption, body weight, water intake, urine creatinine, and GSH, and significant ($p < 0.05$) decreases in urine output, plasma creatinine, urea, TBARS and urine total protein in the treated groups when compared with the toxic control group. This was

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further evident by a significant improvement or reversal of the histopathological alterations of kidney tissues in the groups treated with AOGL. The results of this study indicated that AOGL ameliorated the kidney injury caused by gentamicin in rats. Hence, the extracts have the potential of being used for the management of gentamicin-induced nephropathies.

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1. Introduction

Gentamicin (Gen.) is an antibiotic that is widely used against serious and life-threatening gram-negative bacterial infection. However, the clinical use of gentamicin is limited due to its nephrotoxicity [1]. Gentamicin induces nephrotoxicity via oxidative and nitrosative stress [2]. It also causes nephrotoxicity by inhibiting protein synthesis in renal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure [3]. Gentamicin binds to the phospholipids of the cell membrane of the renal tubules and enters the cells, then it binds to sub-cellular organelles and alters mitochondrial respiration, while small amounts may be taken up by lysosomes [4]. In addition, induction of acute tubular necrosis, glomerular damage and renal inflammation are the major events implicated in gentamicin nephrotoxicity [5,6]. *Ocimum gratissimum* (OG) is an herbaceous plant that belongs to the Labiatae family. The plant is indigenous to tropical areas especially India and West Africa. In Nigeria, it is found in the savannah and coastal areas. It is cultivated in Sri Lanka, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan [7]. It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), and Nimma tulasi (Kannada). In the southern part of Nigeria, the plant is called “effirin-nla” by the Yoruba speaking tribe. It is called “Ahuji” by the Igbos, while in the Northern part of Nigeria, the Hausas call it “Daidoya” [8]. OG has been used extensively in the traditional system of medicine in many countries. In the north east of Brazil, it is used for medicinal, condiment and culinary purposes. The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion [9]. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea [8]. In the savannah areas decoctions of the leaves are used to treat mental illness [10]. OG is used by the Ibos of Southeastern Nigeria in the management of baby’s cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh [11]. Despite all these medicinal values of *O. gratissimum*, there is a paucity of literature on the use of this plant in the treatment of drug-induced kidney injury. Therefore, this study is aimed at investigating the effect of aqueous extract of *Ocimum gratissimum* (AOGL) on markers of renal function in rats following gentamicin administration.

2. Materials and methods

2.1. Drugs and materials

Gentamicin injection 80 mg/2 ml (manufactured by Shanxi Shuguang Pharmaceutical Co., limited) is marketed as an antibiotic drug against a wide variety of pathogenic gram negative and positive bacteria.

Metabolic cages used for this study were fabricated by Central Technological Laboratory and Workshops (CTLW), OAU, Ile-Ife.

2.2. Plant extraction

The plant leaves were collected, washed, air dried under shade and ground into fine powder using a blender. The aqueous extract was obtained by extracting the powdered leaves (323 g) with 3 liters of distilled water in an electric shaker for 48 hours. The extract was filtered through Whatman No. 1 (Whatman International Ltd, Maidstone, UK) paper and evaporated under reduced pressure using a rotary evaporator. The residue (33.60 g) was kept in a bottle with a tight fitting cover until it was needed for the study.

$$\begin{aligned}\text{Extraction yield in \%} &= 33.60/323 \times 100 \\ &= 10.4\%.\end{aligned}$$

2.3. Animal care and management

Sixty-five adult male Wistar rats weighing 150 g–200 g were used for this study; they were obtained from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, and were housed in a plastic cage. The animals were kept inside metabolic cages under normal environmental conditions of light/dark cycle and had free access to standard rodent pellet diet (Caps Feed PLC Osogbo, Nigeria) and water. They were allowed to acclimatize in the laboratory for two weeks before the commencement of the study. The experimental procedures adopted in this study were in strict compliance with the Experimental Animal Care and Use of Laboratory Animals in Biomedical Research, College of Health Sciences, Obafemi Awolowo University, Ile-Ife.

2.4. Experimental design

The rats were divided into 5 groups as follows: Group 1 (the control) consisting of 5 rats received 2 ml/kg distilled water daily

by oral route for the whole period of study (2 weeks). Twenty-four hours after the last administration of distilled water, the rats were sacrificed by cervical dislocation. Group 2 (the toxic control) consisted of 10 rats, each of which received 100 mg/kg/day of gentamicin i.p. for a week. They were left untreated with aqueous extract of *Ocimum gratissimum* (AOGL) for 2 weeks. Five of the rats were sacrificed twenty-four hours after the last administration of gentamicin, while the remaining 5 rats were sacrificed on the 14th day of recovery period. Groups 3, 4 and 5 consist of 5 rats each. They were pre-treated with gentamicin as the Group 2 rats, after which they received 100, 200 and 400 mg/kg/day each of AOGL p.o., respectively, for 14 days. Rats from each group were sacrificed on the 14th day of

2.7.2. Urine creatinine and urea

Urine samples obtained were diluted 1 + 49 with distilled water before 0.1 ml of the diluted urine sample was pipetted into the test tube for creatinine and urea determination.

2.7.3. Protein determination

Protein determination was carried out according to the method of Lowry et al. [13] as described by Holme and Peck [14].

2.8. Creatinine clearance

This was determined using the following equation:

$$\text{Creatinine clearance} = \frac{\text{urine creatinine } (\mu\text{mol/L}) \times (\text{volume of urine (ml) 24 hrs/1440 min})}{\text{plasma creatinine } (\mu\text{mol/L})}$$

treatment with AOGL. Throughout the study period, the body weights of the rats were measured once a week. Their 24-hour food consumption, water intake and urine output were also recorded weekly. Renal creatinine clearance was calculated for all the groups using standard formula ($C = U \times V / P$).

C = clearance, U = urine creatinine concentration,

V = urine flow rate (volume of urine/time),

P = plasma creatinine concentration.

2.5. Measurement of body weight

The body weight of the rats were measured throughout the study period using a digital weighing scale (Camry weighing balance; China) to access the weight gain or loss in each group.

2.6. Measurement of food consumption, water intake and urine volume

The food consumption and water intake of the rats were determined by taking the difference between the previous weight and volumes respectively from the left over after 24 hours. The value obtained was taken as the daily food consumption and water intake of the group. The volumes of water consumed and urine produced by each rat were measured with a measuring cylinder and the food consumption by the digital weighing scale.

2.7. Biochemical assay

About 3 ml of whole blood collected into an EDTA tube was centrifuged at 4000 rpm for 15 min using a Cold Centrifuge (Centurium Scientific, Model 8881, UK). The plasma obtained was analyzed for creatinine, urea and total protein. Similar procedure was applied to the urine sample.

2.7.1. Plasma creatinine and urea

The plasma creatinine concentration was estimated by colorimetric method [12] using the laboratory protocol outlined by Randox Manual/RX Monza CR 510 (standard laboratory kit).

2.9. Measurement of non-enzymatic antioxidants

The kidneys of the rats were carefully excised and weighed. One of the kidneys was homogenized with 10 ml of sucrose solution (0.25 M) using Electric Homogenizer (SI601001). The homogenate was centrifuged at 3000 rpm for 20 minutes and the supernatant was collected for the assessment of glutathione and TBARS activities.

2.9.1. Estimation of reduced glutathione (GSH) and estimation of thiobarbituric acid reactive substance (TBARS)

Reduced glutathione (GSH) was measured by the method of Beutler et al. [15] and TBARS level was determined by the method of Ohkawa et al. [16].

2.10. Histopathological studies

The tissues were fixed in 10% formo-saline to prevent putrefaction and autolysis, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (H&E) for histological analysis. The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photomicrographs were taken with a Leica DM 750 Camera at $\times 400$ magnification.

2.11. Statistical analysis

The results obtained were expressed as mean \pm SEM. The data were analyzed using one-way ANOVA followed by Newman-Keuls test using Graph Pad 5.03 (Graph Pad Software Inc., CA, USA). The results were considered significant when $p < 0.05$.

3. Results

3.1. Food consumption and body weight

The food consumption and body weight of the control group increased significantly ($p < 0.05$) throughout the study period (Table 1). An insignificant increase in food consumption and body weight was observed in the gentamicin treated rats when

Table 1 – Effect of aqueous extract of *Ocimum gratissimum* on food consumption (g) and body weight in gentamicin-induced nephrotoxicity in rats.

	Food consumption			Body weight		
	Pre-treatment	Gentamicin treatment	14 days' treatment	Pre-treatment	Gentamicin treatment	14 days' treatment
Control	18.00 ± 1.26	21.40 ± 2.91	26.40 ± 0.68*	204.0 ± 3.55	212.6 ± 3.89	226.0 ± 2.92*†
Toxic control	27.00 ± 2.00	33.60 ± 5.27	–	188.0 ± 12.41	195.6 ± 11.74	–
Toxic recovery	15.00 ± 0.76	14.40 ± 1.63	26.00 ± 1.4*†	94.00 ± 1.73	101.2 ± 2.44	136.0 ± 4.14*†
100 mg AOGL	11.00 ± 1.79	14.80 ± 2.15	21.00 ± 2.04*†	98.00 ± 3.86	87.40 ± 5.14	132.8 ± 10.00*†
200 mg AOGL	12.60 ± 2.56	17.80 ± 1.16	24.40 ± 3.14*†	117.4 ± 9.05	118.8 ± 8.62	142.0 ± 6.61*†
400 mg AOGL	15.60 ± 1.69	17.20 ± 2.06	20.80 ± 1.77*†	92.20 ± 4.34	96.60 ± 6.38	129.8 ± 7.94*†

Values are given as mean ± SEM. * = Significantly different from pre-treatment values. † = Significantly different from toxic control (p < 0.05). AOGL, aqueous extract of *Ocimum gratissimum*.

compared with its pre-treatment values during the 8-day treatment with gentamicin (Table 1). However, a significantly higher food consumption and body weight was recorded in the rats treated with AOGL after gentamicin administration when compared with their pre-treatment values and during gentamicin treatment (p < 0.05) (Table 1). The food consumption and body weight of the toxic recovery group were significantly higher (p < 0.05) when compared with its pre-treatment values and after the administration of gentamicin.

3.2. Water intake and urine volume

The urine volume of the toxic control group was significantly higher (p < 0.05) than that of its pre-treatment value (Table 2). This increase was accompanied by a reduction in water intake, which was significantly lower (p < 0.05) than its pre-treatment value. At the end of the 14-day treatment with AOGL, the water intake of the treated rats was significantly higher (p < 0.05) than that of their pre-treatment values and 8-day gentamicin treatment period except for rats treated with 400 mg/kg/day of AOGL, which showed no significant difference in water intake compared to its pre-treatment value and during gentamicin administration (Table 2). The experimental groups had a significantly higher urine volume (p < 0.05) during the 8 days of treatment with gentamicin when compared with their pre-treatment values. However, their urine volume dropped significantly at the end of treatment with AOGL (p < 0.05). Similarly, rats that were allowed a 2-week recovery period after

gentamicin treatment had a significantly lower (p < 0.05) urine volume compared to the value obtained during gentamicin administration (Table 2).

3.3. Plasma and urine creatinine, urea and total protein

A significant increase in the plasma creatinine and urea concentrations was observed in the toxic control and the recovery group when compared with the control rats (p < 0.05) (Figs. 1 and 2). The plasma creatinine and urea concentrations of the rats treated with AOGL were found to be significantly lower (p < 0.05) than that of the toxic control group. The concentrations of urea and creatinine in the plasma of the treated rats were not significantly different from that of the control groups, but the rats that were allowed a 2-week recovery period after gentamicin treatment had a significantly higher (p < 0.05) plasma creatinine concentration when compared to the control rats (Figs. 1 and 2).

The plasma total protein of the toxic control group and the AOGL treated group reduced significantly (p < 0.05) when compared with the control group (Fig. 3). Similarly, the toxic recovery group had significantly lower (p < 0.05) plasma total protein concentration compared to the control group (Fig. 3).

The urine creatinine concentration of the toxic control was observed to be significantly lower (p < 0.05) than that of the control group (Fig. 4). However, the urine creatinine concentration of the treated rats and recovery group was significantly higher (p < 0.05) than that of the toxic control, but significantly lower (p < 0.05)

Table 2 – Effect of aqueous extract of *Ocimum gratissimum* on water consumption (ml) and urine volume (ml) in gentamicin-induced nephrotoxicity in rats.

	Water consumption			Urine output		
	Pre-treatment	Gentamicin treatment	14 days' treatment	Pre-treatment	Gentamicin treatment	14 days' treatment
Control	31.00 ± 2.12	30.60 ± 9.51	29.20 ± 3.40	9.20 ± 1.02	10.90 ± 0.95	9.40 ± 1.45
Toxic control	17.40 ± 2.16	13.00 ± 0.95	–	6.00 ± 0.89	9.50 ± 0.80*	–
Toxic recovery	10.00 ± 1.38	13.80 ± 1.59	33.50 ± 4.29*	5.00 ± 0.45	8.90 ± 1.07*	3.12 ± 1.32†
100 mg AOGL	9.400 ± 0.60	15.00 ± 1.92	29.00 ± 3.14*†	3.90 ± 0.58	7.60 ± 0.37*	4.25 ± 1.74†
200 mg AOGL	11.20 ± 0.97	16.60 ± 2.38	28.00 ± 3.56*†	3.90 ± 0.43	7.00 ± 1.19*	4.60 ± 1.45†
400 mg AOGL	11.60 ± 1.86	23.60 ± 2.75	30.40 ± 4.57	4.50 ± 0.54	8.10 ± 1.22*	4.20 ± 0.97†

Values are given as mean ± SEM. * = Significantly different from pre-treatment values. † = Significantly different from toxic control (p < 0.05). AOGL, aqueous extract of *Ocimum gratissimum*.

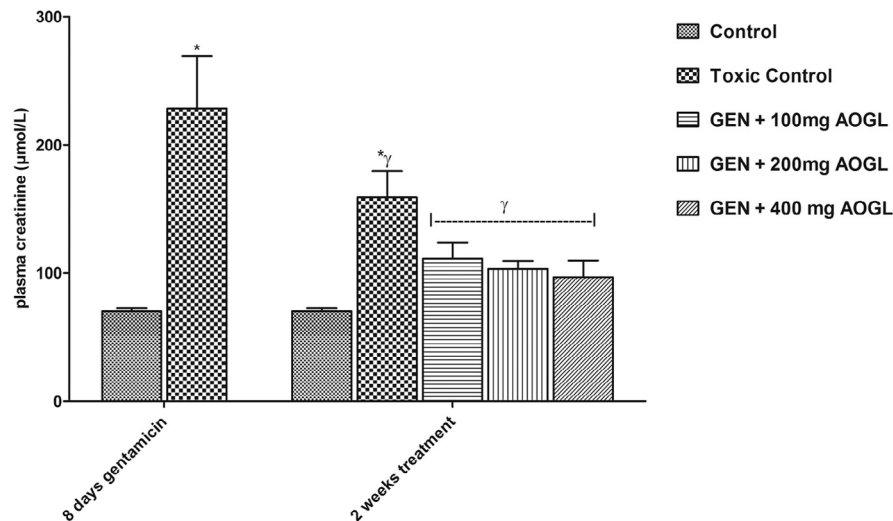


Fig. 1 – Effect of aqueous extract of *Ocimum gratissimum* on plasma creatinine level in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control. γ = Significantly different from toxic control ($p < 0.05$).

than that of the control group (Fig. 4). The urine concentration of urea in the toxic control group was significantly higher ($p < 0.05$) than that of the control group (Fig. 5). However, AOGL treated groups and the toxic recovery group had a significantly lower ($p < 0.05$) urine concentration of urea when compared with the toxic control group, which was not significantly different from the control rats (Fig. 5). The urine total protein concentration of the toxic control was significantly higher ($p < 0.05$) when compared to the control group (Fig. 6). The toxic recovery group had urine total protein concentration which was significantly lower ($p < 0.05$) than that of the control and toxic control. The group treated with 100 mg/kg/day of AOGL had a significantly higher ($p < 0.05$) urine total protein concentration when compared to the toxic recovery group, which was significantly lower ($p < 0.05$) than that of the toxic control group (Fig. 6). The plasma total

protein concentration of rats treated with 400 mg/kg/day of AOGL was significantly higher ($p < 0.05$) than that of the toxic recovery group.

3.4. Relative kidney weight, TBARS and GSH

A significant increase in the relative kidney weight was observed in the toxic control group, toxic recovery group and the AOGL treated rats when compared with the control group ($p < 0.05$) (Fig. 7).

The TBARS and GSH of the toxic control group were significantly higher ($p < 0.05$) when compared with the control group, while the TBARS and GSH of the toxic recovery group and rats treated with AOGL decreased significantly ($p < 0.05$) when compared with the toxic control group (Figs. 8 and 9).

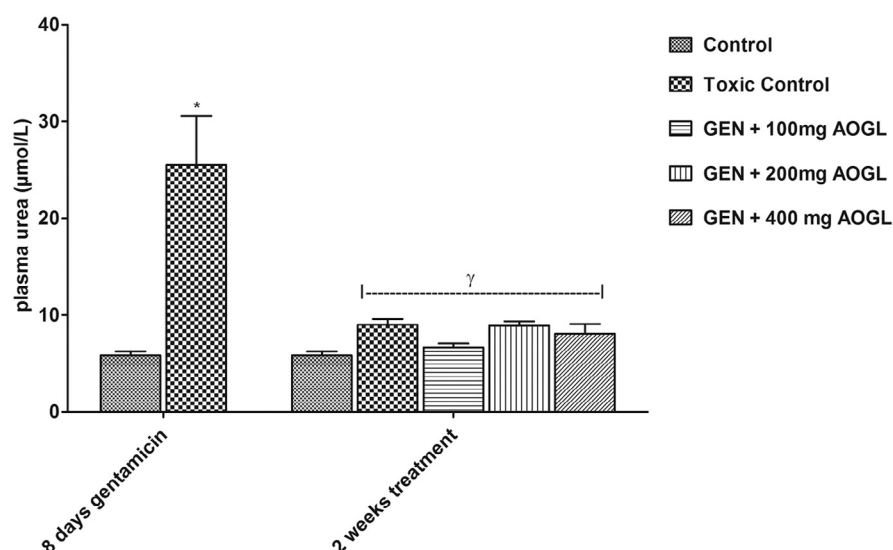


Fig. 2 – Effect of aqueous extract of *Ocimum gratissimum* on plasma urea in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control. γ = Significantly different from toxic control ($p < 0.05$).

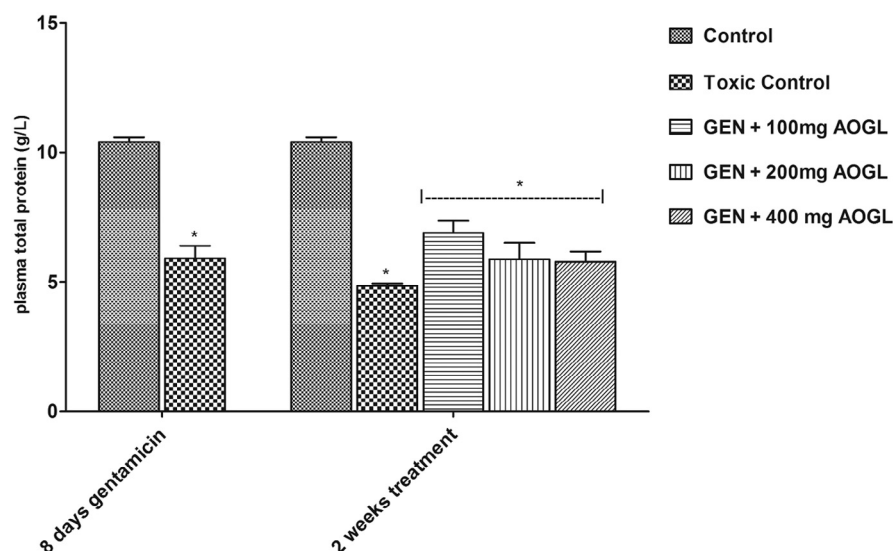


Fig. 3 – Effect of aqueous extract of *Ocimum gratissimum* on plasma total protein in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control ($p < 0.05$).

3.5. Creatinine clearance

A significant decrease in the renal creatinine clearance was observed in the toxic control group, toxic recovery group and the AOGL treated rats when compared with the control group ($p < 0.05$) (Fig. 10).

3.6. Histological examination

The photomicrograph of the toxic control showed decreased cellularity in the glomeruli (G), loss of cellular constituents (double arrow) and densely eosinophilic cast in the lumen of the tubules (dashed arrow) resulting in atrophy and severe cloudy swelling of the distal convoluted tubule (yellow arrow)

when compared with the control, which showed intact renal corpuscles with normal appearing glomeruli (G) and tubules (T), as well as intact Bowman's space (black arrow) and epithelial lining of Bowman's capsule (arrowhead) (Figs. 11 and 12), while the toxic recovery group showed marked decrease cellularity in the glomeruli, loss of cellular constituents of tubules (double arrow), densely eosinophilic ('colloid') cast in the lumen of some tubules (dashed arrow), cloudy swelling/inflammation of the proximal and distal convoluted tubules (yellow arrow) (Fig. 13).

The photomicrographs of the kidneys of rats treated with graded doses of AOGL revealed that the glomeruli (G) and surrounding Bowman's space (black arrow) are mostly intact. There is slight loss of cellular constituents of tubules and no

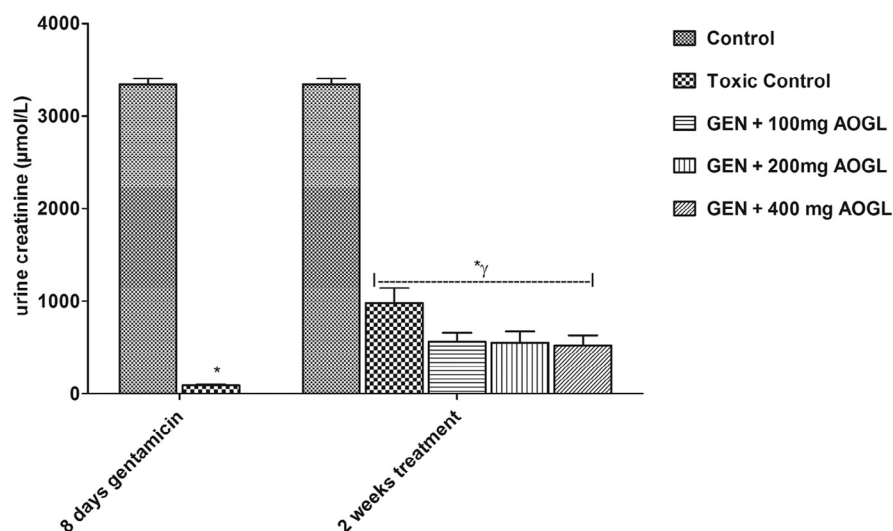


Fig. 4 – Effect of aqueous extract of *Ocimum gratissimum* on urine creatinine in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control. γ = Significantly different from toxic control ($p < 0.05$).

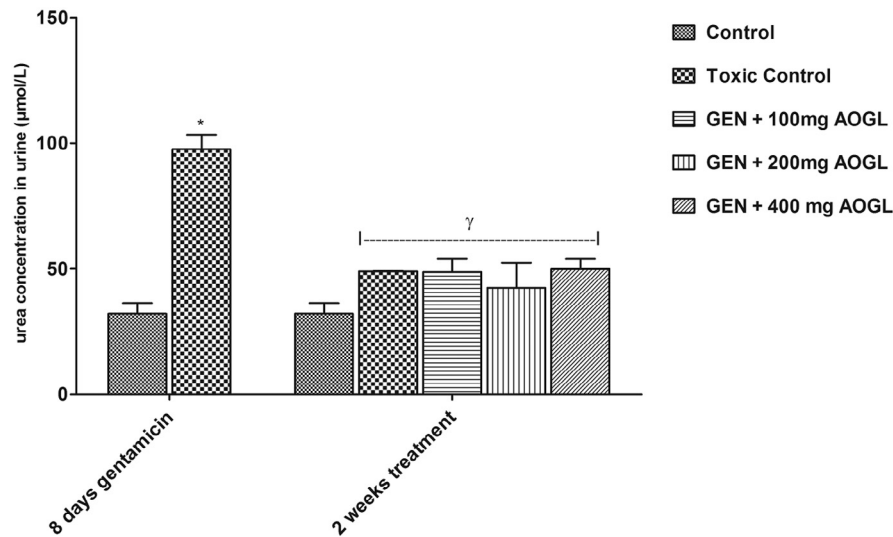


Fig. 5 – Effect of aqueous extract of *Ocimum gratissimum* on urine urea in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control. γ = Significantly different from toxic control ($p < 0.05$).

eosinophilic ('colloid') casts are observed in the lumen of tubules (Figs. 14–16).

4. Discussion

In this study, an insignificant increase in food consumption and body weight was observed in the gentamicin treated rats when compared with its pre-treatment values. This is in contrast with the findings of Erdem et al. [4] and El-Zawahry and Abu El Kheir [17], who reported that gentamicin administration caused reduction in food intake and body weight.

Morley et al. [18] reported that there was a physiological decline in food intake with aging which is multi-factorial and involves both peripheral and central mechanisms. Altered hedonic qualities of food occur due to alterations in taste and, more particularly, smell with aging. They further explained that a decline in adaptive relaxation of the fundus of the stomach and an increased rate of antral filling appear to play a role in the early satiation seen in many aged persons. Cholecystokinin levels are increased with aging and aged persons are more sensitive to the satiating effects of this gut hormone. For these reasons, the increase in food consumption and body weight that was observed in the gentamicin treated rats may be due to the wide differences in the age and body weight of the rats

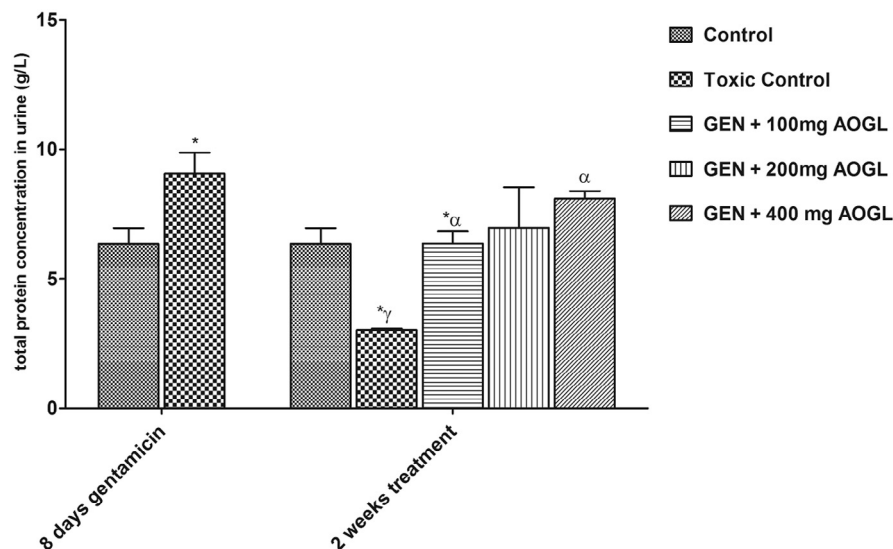


Fig. 6 – Effect of aqueous extract of *Ocimum gratissimum* on urine total protein in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control. γ = Significantly different from toxic control ($p < 0.05$). α = Significantly different from toxic recovery group ($p < 0.05$).

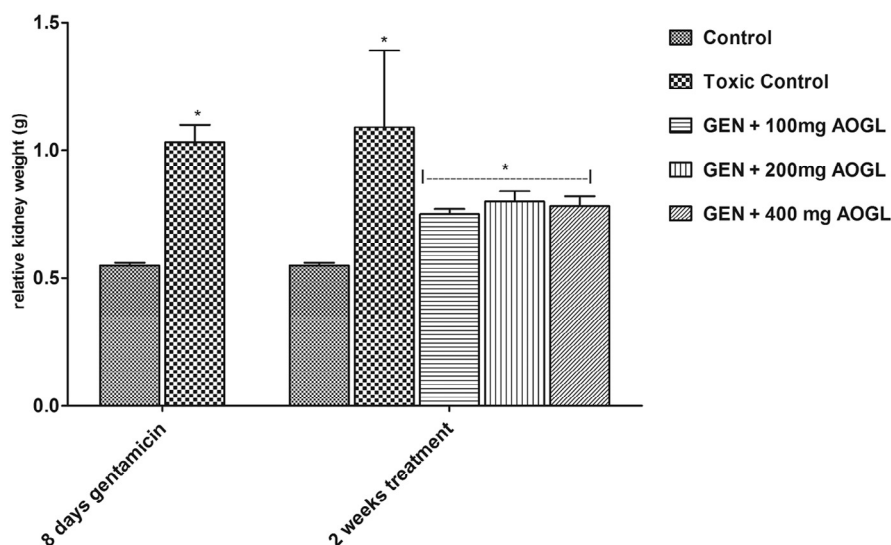


Fig. 7 – Effect of aqueous extract of *Ocimum gratissimum* on the relative kidney weight in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control ($p < 0.05$).

used in this study (100–150 g) compared with that of the rats used by El-Zawahry and Abu El Kheir [17] (210–230 g).

Gentamicin has been reported to cause a decrease in the number of cytokines cells (such as CD3(+), CD56(+), CD3(+), CD8(+), CD3(+), CD56(+)) with some cytotoxicity [19]. Cytokines are immune-regulatory peptides released in response to chronic inflammation, infections, injuries, and neoplasms [20]. Cytokines are potent anorectic agents, they decrease serum albumin, induce lipolysis, produce muscle protein breakdown, and induce nitrogen loss [20]. These have a variety of effects that can lead to severe malnutrition [20]. Hence, the significant increase in food consumption and body weight that was observed in the toxic recovery group when compared with the pre-treatment values is an indication of the ability of gentamicin to decrease the numbers of cytokines in the rats' body.

Study has shown that administration of AOGL in rats caused significant histological changes in the intestines, revealing the presence of increased villi and larger goblets cells [21]. The increase in the numbers of villi in the intestine facilitates increase in nutrient absorption due to increase in the surface area of the intestine [22]. Furthermore, AOGL enhances anti-diarrhea effect by inhibiting intestinal motility, partly via muscarinic receptor inhibition [23], thereby facilitating nutrient absorption in the intestine. The significant increase in body weight that was seen in the experimental groups treated with AOGL may be as a result of AOGL-induced inhibition of the intestinal motility, together with increase in the number of villi of the intestine and larger goblets cells, which favor rapid absorption of nutrient from the gastro-intestinal tract. However, this is not in agreement with the report of Ajibade et al. [24], who

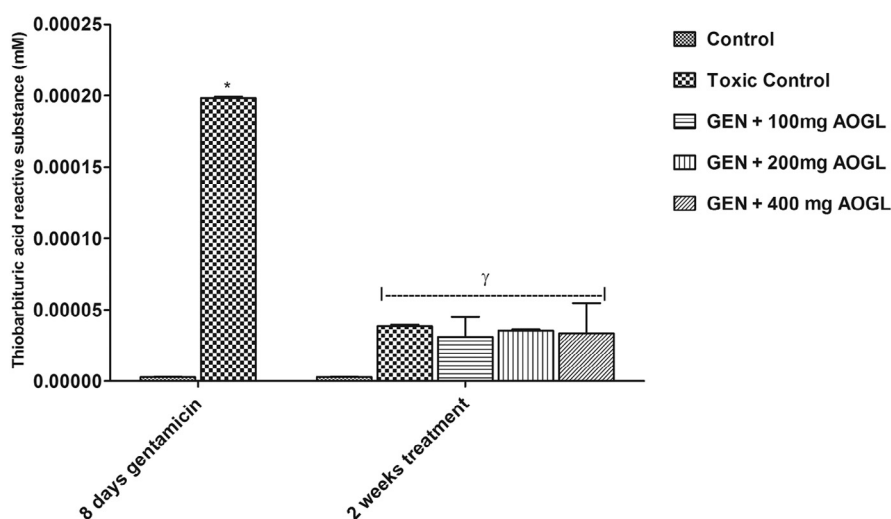


Fig. 8 – Effect of aqueous extract of *Ocimum gratissimum* on TBARS in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control. γ = Significantly different from toxic control ($p < 0.05$).

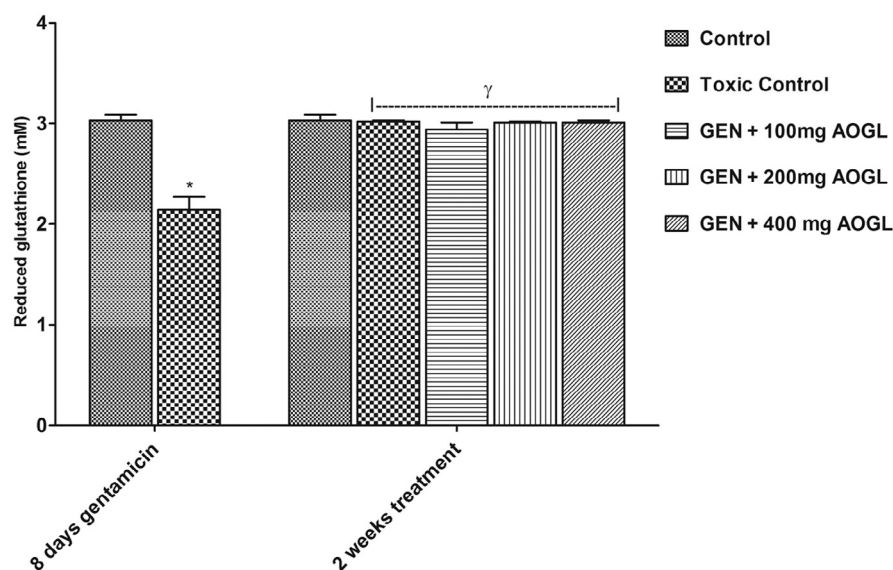


Fig. 9 – Effect of aqueous extract of *Ocimum gratissimum* on GSH in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control. γ = Significantly different from toxic control ($p < 0.05$).

reported that AOGL administered at different doses caused a significant decrease in the body weight. This disparity may have resulted from the differences in the doses administered to the rats. While Ajibade et al. used 400, 800, 1600 and 3200 mg/kg/bw, in this study 100, 200 and 400 mg/kg/bw were used. Another possible explanation for the significant increase in food consumption and body weight that was observed in the groups treated with AOGL could be due to its hypoglycemic property attributed to flavonoids and other phytochemical constituents [25]. The extract also contains major mineral elements, e.g. calcium, chloride, manganese, magnesium, zinc and potassium, which might also play a contributory role in enhancing its hypoglycemic property [25]. Administration of the AOGL

caused a significant reduction in plasma glucose level in streptozotocin induced diabetic rats [26]. Decrease in the plasma glucose level is said to inhibit the satiety center, thus activating the feeding center to increase appetite leading to increase in food intake [27]. Leptin, a cytokine-like peptide hormone that is secreted from adipose cells deficiency, has been found to be responsible for the hyperphagia and obesity of the genetically obese mouse [28]. Hence, the increase in food consumption and body weight of rats treated with AOGL is indicative of its ability to reduce the secretion of leptin. This requires further study.

The significant increase in urine volume that was observed in the toxic control rats without a corresponding increase

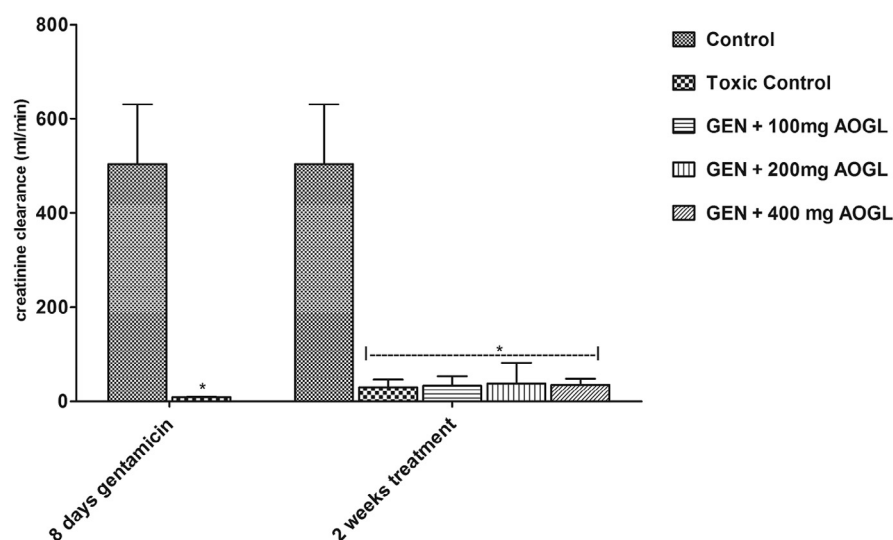


Fig. 10 – Effect of aqueous extract of *Ocimum gratissimum* on creatinine clearance in gentamicin-induced kidney injury in rats. Values are given as mean \pm SEM. * = Significantly different from control. γ = Significantly different from toxic control ($p < 0.05$).

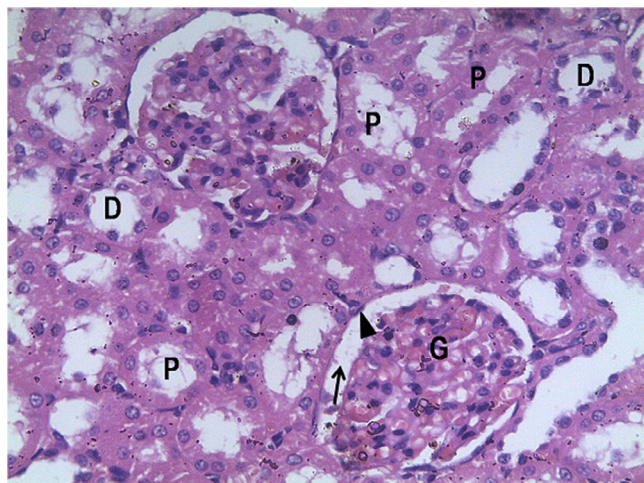


Fig. 11 – Photomicrograph of the kidney of the control (CN). H&E $\times 400$. Intact renal corpuscles with normal appearing glomeruli (G) and tubules (T), including the PCT (P) and DCT (D), are seen in control, as well as intact Bowman space (black arrow) and epithelial lining of Bowman capsule (arrowhead).

in water intake may lead to dehydration and severe depletion of the major electrolytes of their body fluid with the attendant consequences on the cardiovascular system. This observed change in urine volume is in accordance with the finding of El-Zawahry and Abu El Kheir [17], who reported that gentamicin administration to rats induced polyuria. However, the water intake of the toxic recovery group and rats treated with AOGL increased significantly without a corresponding increase in urine output. This could be due to an attempt by the

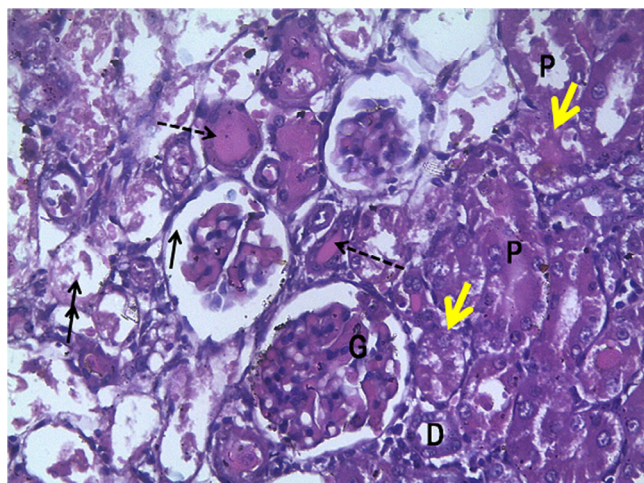


Fig. 12 – Photomicrograph of the kidney of the toxic control (TX). H&E $\times 400$. Decreased cellularity in the glomeruli, loss of cellular constituents of tubules (double arrow), densely eosinophilic ('colloid') cast in the lumen of some tubules (dashed arrow) resulting in atrophy, loss of epithelial cells and severe cloudy swelling/inflammation of the distal convoluted tubules (yellow arrow) are observed in the toxic control group.

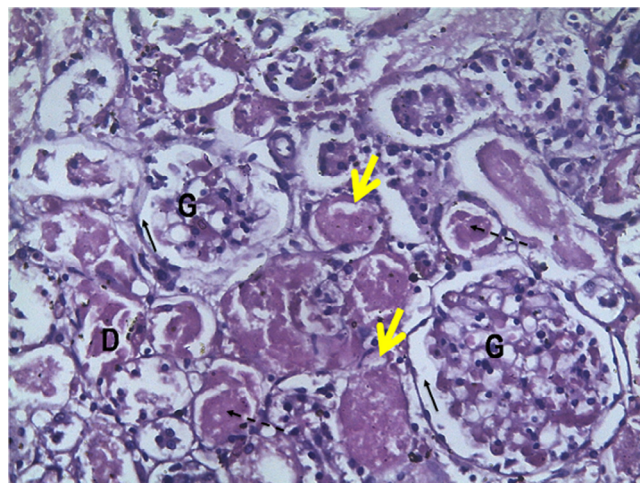


Fig. 13 – Photomicrograph of the kidney of toxic recovery group. H&E $\times 400$. Marked decreased cellularity in the glomeruli, loss of cellular constituents of tubules (double arrow), densely eosinophilic ('colloid') cast in the lumen of some tubules (dashed arrow), and cloudy swelling/inflammation of the proximal and distal convoluted tubules (yellow arrow) are seen in the toxic recovery group.

rats to restore the body fluid volume resulting from dehydration that followed gentamicin treatment or increased osmolarity caused by depletion of their body fluid. This leads to stimulation of osmoreceptor cells which activate the thirst center in the hypothalamus to facilitate increase in water intake.

The significant increase in relative kidney weight that was observed in the toxic control group and toxic recovery group may be due to inflammation in response to injury caused by this drug. This is corroborated by the photomicrograph of the

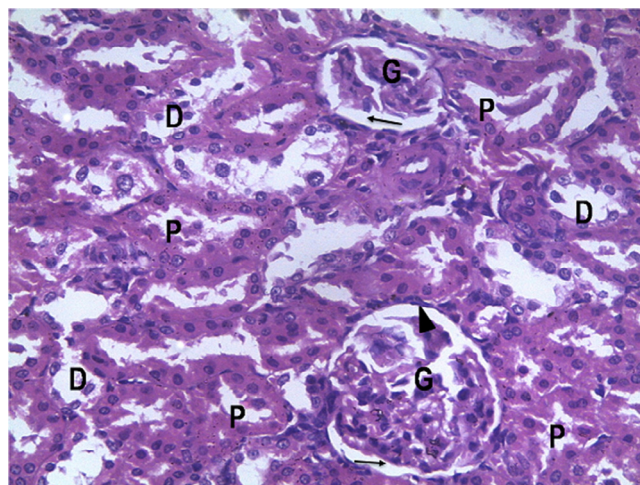


Fig. 14 – Photomicrograph of the kidney of 2 weeks' treatment with 100 mg/kg/day of AOGL. H&E $\times 400$. Glomeruli (G) and surrounding Bowman's space (black arrow) are mostly intact. There is slight loss of cellular constituents of tubules and no eosinophilic ('colloid') casts are observed in the lumen of tubules.

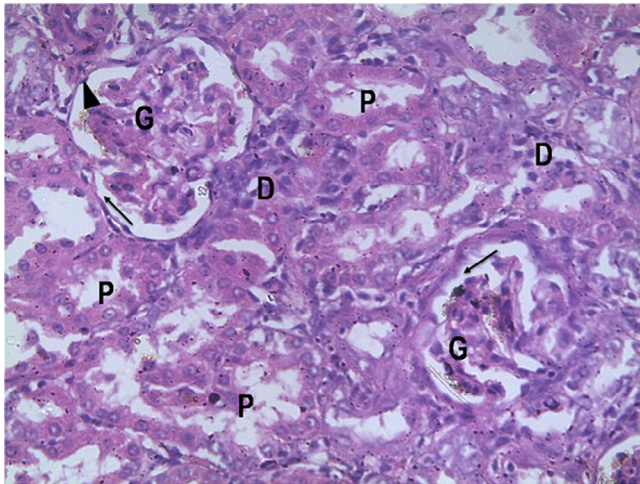


Fig. 15 – Photomicrograph of the kidney of 2 weeks' treatment with 200 mg/kg/day of AOGL. H&E ×400. Glomeruli (G) and surrounding Bowman's space (black arrow) are mostly intact. There is slight loss of cellular constituents of tubules and no eosinophilic ('colloid') casts are observed in the lumen of tubules.

kidney of this group which showed severe cloudy swelling of the renal tubules. Similar observation was made by El-Zawahry and Abu El Kheir [17]. Also, a significant increase in relative kidney weight was observed in the groups treated with AOGL. This finding is not in agreement with Sahouo et al. [29], who reported that AOGL exhibited an anti-inflammatory effect on the kidney of rats. The observed discrepancy is likely attributable to the duration of administration of AOGL.

The plasma creatinine and urea concentrations of the toxic control group were significantly higher than that of the control rats. The creatinine and urea concentrations in

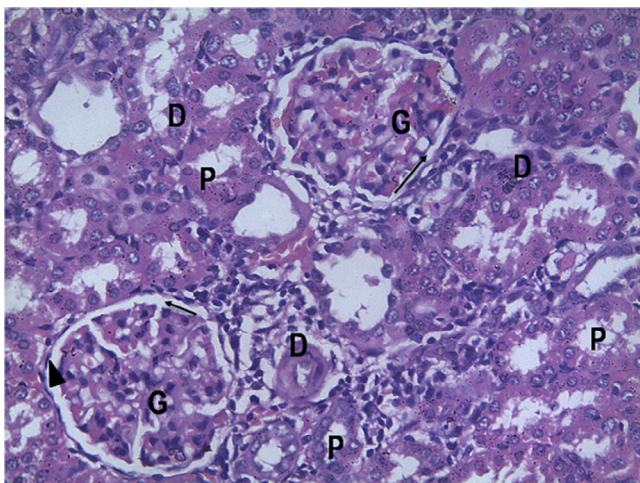


Fig. 16 – Photomicrograph of the kidney of 2 weeks' treatment with 400 mg/kg/day of AOGL. H&E ×400 (right). Glomeruli (G) and surrounding Bowman's space (black arrow) are mostly intact. There is slight loss of cellular constituents of tubules and no eosinophilic ('colloid') casts are observed in the lumen of tubules.

the urine reduced significantly in this group compared to the control. These observed changes are in accordance with the report of Pedrazha-Chaverri et al. [30], who reported that administration of gentamicin caused marked impairment in renal function. The decrease in urine creatinine is a further evidence of reduced ability of the renal tubules to extract and remove creatinine from the plasma of the toxic control rats. The elevated concentration of plasma urea observed may have resulted from the inability of the glomeruli to filter urea. A significant decrease toward normal plasma level of creatinine and urea that was observed in the toxic recovery group is an indication of tubular regeneration resulting from the release of reparative and prosurvival factors from the distal tubular cells [31]. The marked decrease in plasma creatinine and urea in AOGL treated groups is in conformity with report of other researchers, who reported that AOGL decreased serum urea, creatinine, uric acid, urine volume, urinary protein and increased urine creatinine level in cisplatin induced nephrotoxicity in rats [32,33].

A significant decrease in renal creatinine clearance that was observed in the toxic control and toxic recovery group is in agreement with the report of Raheem et al. [34] and Bibu et al. [35] that gentamicin administration to rats reduced their glomerular filtration rate and renal blood flow, resulting from a rise in renal vascular resistance or damage to the glomerular capillary endothelium leading to impairment in glomerular function. Similarly, a significant decrease in renal creatinine clearance was observed in rats treated with AOGL when compared to the control. However, the creatinine clearance of these rats increased when compared to the toxic control group. This is an indication of improved blood flow to the kidney as well as repair of kidney tissue damage caused by gentamicin.

A significant increase in the urinary total protein was observed in the toxic control group. It is an evidence of inflammation of the glomerular capillaries. This observed change is in accordance with the result of El-Zawahry and Abu El Kheir [17], who reported that gentamicin administration to rats induced proteinuria. In contrast, rats treated with AOGL showed a decrease in urine protein. This may be due to the antioxidant properties of AOGL since it has been found that reactive oxygen species (ROS) may be involved in the impairment of glomerular filtration [2].

A significant increase in the TBARS level and significant decrease in GSH level that were observed in the toxic control group are evidence of increased lipid peroxidation, which is an indicator of oxidative stress. This observed change is in conformity with the report of Reddy et al. [36], who reported that gentamicin administration caused oxidative damage to the kidney. Also a significant decrease in the TBARS level and increase in GSH level were observed in the toxic recovery group when compared with the control group. This finding is an indication of the release of reparative and prosurvival factors from the distal tubular cells [37]. Furthermore, a significant decrease in the TBARS and increase in GSH level were observed in the AOGL treated group when compared with the control group. This is in agreement with Reddy et al. [36], who reported that simvastatin increased GSH and decreased TBARS in gentamicin induced kidney injury. The change observed may be due to the anti-oxidant activities of AOGL.

5. Conclusion

This study demonstrated the nephron-restorative effect of *O. gratissimum* in gentamicin induced nephrotoxicity in rats. The ameliorative effect of *O. gratissimum* is evident by a remarkable restoration of markers of renal function such as protein, urea, creatinine levels, creatinine clearance and antioxidant enzymes in gentamicin treated rats.

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